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Current and Novel Strategies for Biomarkers Detection in Neurodegenerative Diseases: Future Graphene-Based Applications

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Abstract

Scientific progress has significantly reduced pandemic death causes, thus progressively increasing life expectancy. This achievement forced researchers to face the main problem related to an aging population: the occurrence of neurodegenerative disorders (NDDs). Unfortunately, today only symptomatic drugs are available to delay the disease progression of NDDs. Moreover, the specificity and selectivity of the current clinical diagnosis does not allow an early disease detection for a prompt clinical intervention before symptoms onset. Therefore, part of the research is now focusing on two main aspects: i) find out new selective biomarkers, ii) detect biomarkers in body fluids with more sensitive and specific technological tools. In particular, in the last decade graphene has raised great interest thanks to its unique chemical properties, easy availability, biocompatibility and low cost for the synthesis of electrochemical and optical sensors-biosensors.
Here we report some of the most relevant new biomarkers under investigation and the new graphene tools that could be potentially used to improve NDDs early diagnosis. Despite further investigations are needed before a useful clinical employment of such new nanomaterials, promising positive results are expected in the next future.

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Backgrounds

The mean age of the world's population is increasing and, according to the World Population Prospects, from 2015 to 2030 it is estimated that elderly people will raise by 56% ("World Population Ageing 2015" 2015). Scientific progress has significantly reduced pandemic death causes like infections, thus increasing life expectancy to around 80 years. Although cardiovascular diseases and cancer still remain the first causes of death, the continuous increase in life expectancy forced researchers to face the main problem related to an aging population: the occurrence of neurodegenerative disorders (NDDs). NDDs comprise more than 600 diseases (Summers et al. 2017) characterized by a progressive loss of neurons in central/peripheral nervous system. The etiology and the areas affected define a variety of NDDs with different clinical outcomes. The most prevalent disease is Alzheimer's disease (AD), a dementia disorder with an estimated number of 44 million people affected worldwide (Prince et al. 2016), followed by Parkinson's disease (PD, incidence rate of 4.5–19 per 100 000 population per year, ("Public Health Challenges WHO Library Cataloguing-in-Publication Data" 2018)).

The long list of NDDs also includes autoimmune and motor neuron disorders such as, respectively, multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). Indeed, the third common type of NDD is MS, an autoimmune pathology that nowadays affects around 2.5 million people in the world ("Public Health Challenges WHO Library Cataloguing-in-Publication Data" 2018).

The most common NDD is dementia, if we consider that about every three seconds one person is developing this disorder. Moreover, it has been calculated that there are 9.9 million new cases of dementia each year worldwide with a predicted higher percentage in low and middle-income countries (Figure 1) (Martin Prince et al. 2015). Following the WHO survey, a 60% increase of AD and dementia cases is expected to occur by 2030.

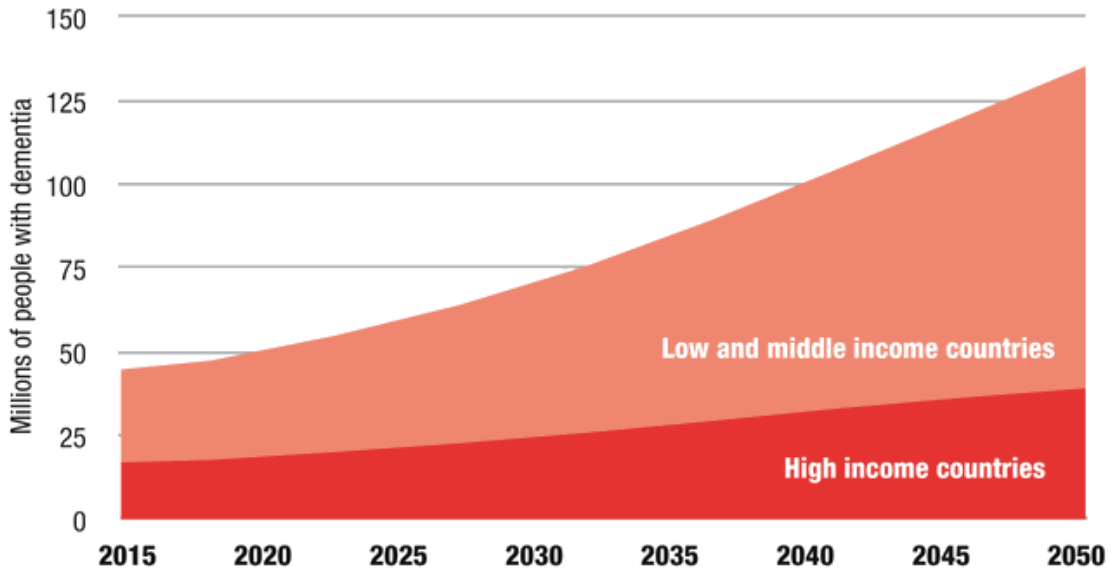

Figure 1. Modified by World Alzheimer Report 2015. Number of people with dementia in low, middle and high income countries.

Neurodegenerative Diseases

Current Methods of Diagnosis

Currently, only symptomatic drugs are available for patients suffering from NDDs, in order to delay the disease progression. Unfortunately, the progressive motor and/or cognitive deficits impair patients' activity of daily living and individual autonomy, resulting in the need of

continuous assistance for everyday life and medications. Thus, the cost of NDDs goes well beyond the direct health cost, i.e. cost mainly borne by the health sector, as these diseases have devastating economic and social impact on both patients themselves and their family's caregivers. To face such a devastating situation selective and effective treatments are urgently needed. In this context, there are two big challenges that researchers have to handle: i) to identify the etiology (ies) of the diseases and the mechanisms of progression of the pathology (ies); ii) to find a timely and early diagnosis for a prompt clinical intervention before symptoms appearance.

Clinical diagnosis currently occurs after the appearance of symptoms, when available therapies are ineffective to cure the disease, and medications can only delay its progression. An early diagnosis preceding the fully symptomatic stage could allow interventions aimed to successfully maintain good levels of physical and mental activity and to delay symptoms manifestation. Nowadays, both AD and PD diagnoses are achieved too late, when symptoms begin to interfere with everyday life. In the case of AD, clinical diagnosis is mainly based on memory impairment, language problems, spatial orientation, executive dysfunction (making decisions, planning, concentration, attention), emotional and social impairment. Similarly, the typical motor symptoms considered fundamental for PD diagnosis are four: tremor, bradykinesia, rigidity, and postural instability. These clinical features can be accompanied by neuropsychiatric disturbances that involve cognitive, mood and behaviour alterations. In the case of MS, 85% of the cases start as clinically isolated syndrome (CIS) with autonomic, visual, motor and sensory problems as the most common symptoms (Tsang and Macdonell 2011).

In all these cases, a proper clinical diagnosis is also supported by the medical and family history of the patient. However, clinical diagnosis requires to be complemented by a series of analyses, including imaging and biochemical assessments, specific for each NDD, in order to unequivocally identify the disease.

Imaging Approaches

Although the localization of early neuronal loss in the brain is different for each NDD (hippocampus in AD, substantia nigra in PD, and striatum in Huntington Disease-HD), the toxic accumulation of misfolded proteins is a common sign. Specific protein accumulations are the typical post-mortem signs found in the brain of patients. In particular, β-amyloid plaques and abnormal total tau proteins (t-tau), are frequently observed in AD, α-synuclein aberrant protofibrils in PD and mutant huntingtin accumulation in HD. Moreover, cerebral lesions generated by the accumulation of these proteins can be visualized by imaging techniques in vivo, supporting a correct NDD diagnosis (Zhang et al. 2017; Reitz and Mayeux 2014). Magnetic resonance imaging (MRI), functional MRI (fMRI) and positron emission tomography (PET) are validated techniques used as supportive methods to confirm the diagnosis.

3D images of the brain physical structures generated by MRI have been demonstrated to identify atrophy in the medial temporal lobe of late-onset AD patients and in the posterior cortex (occipital lobes, posterior cingulate and precuneus) of early onset patients (Karas et al. 2007). Even if certain degree of discriminative diagnostic power can be attributed to this technique, such cerebral changes are not specific to AD, as they can also occur in other NDDs and in normal elderly people. In PD diagnosis, MRI became more accurate over time thanks to the iron-sensitive T2* technique able to reveal macroscopic nigral changes (dopaminergic deficits) present in PD patients with respect to normal individuals. However, despite MRI usefulness in measuring brain atrophy, a proper differential diagnosis is not possible due to structural lesions associated with other forms of parkinsonism (Pagano, Niccolini, and Politis 2016).

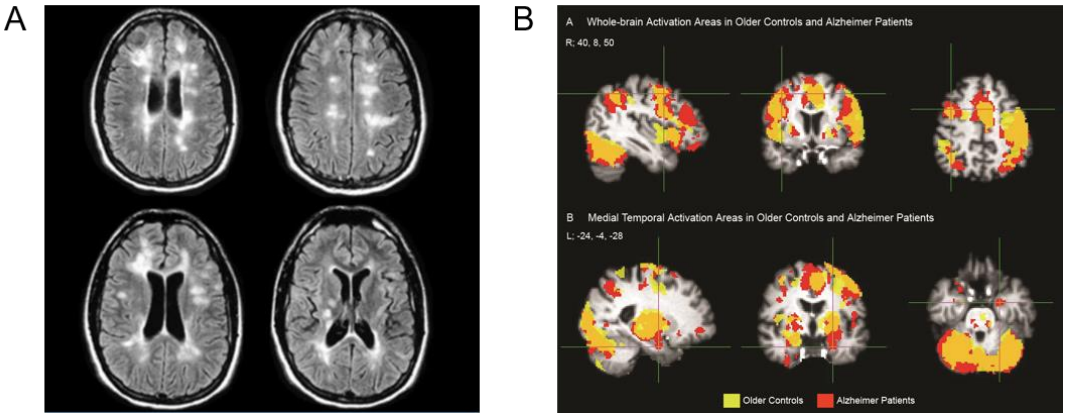

Figure 2. A) MS plaques of a 62 year-old woman with MS (moderate cognitive dysfunction) (image: http://casemed.case.edu/clerkships/neurology/Web Neurorad/MSAdvancedCourse.htm). B) fMRI activity in brain regions during face-name stimuli in patients with AD compared to healthy controls (Kivistö, Soininen, and Pihlajamaki 2014).

MRI tool is also the most relevant, sensitive and non-invasive tool currently employed in the diagnosis of MS (Figure 2.A). Since it is a demyelinating disease caused by an autoimmune response against the central nervous system, scar tissue formation and cerebral lesions can be easily visualized. However, as in the case of AD and PD, a correct diagnosis cannot be based only on MRI. It should be considered that a specific correlation between the brain damages and the clinical symptoms is not always possible and that some areas resembling MS damages are also present during physiological aging. Thus, evoked potentials measures are considered in parallel to detect response deficits after specific brain stimulation. In this way, the slower electrical conduction due to the demyelination process can be detected in areas such as visual, brainstem auditory and somatosensory regions (Ghasemi, Razavi, and Nikzad 2017).

fMRI instead measures and evaluates brain activity based on changes associated with blood flow (Figure 2.B). For example, a decreased activity (corresponding to a recorded decrease blood flow) can be assessed in the medial temporal lobe, parietal lobe and hippocampal areas of AD patients compared to controls during a cognitive task (Golby et al. 2005; Dickerson et al. 2005; Celone et al. 2006). However, inter- and intra-individual variability limits the use of fMRI in the differential diagnosis.

Another very useful technique is PET, which uses cellular metabolism to detect brain dysfunction (Figure 3). Radioactive compounds are administered at a low dose to the patients and can be conjugated to active and tropic and biological molecules. When the tracer is released, the emission of gamma-rays by positron electron collisions is registered, showing low energy intensity in Aβ plaque deposits and neurodegeneration areas. Several radiotracers have been used in AD diagnosis, such as 2-(1-{6-[(2-[F-18]fluoroethyl)(methyl)amino]-2-naphthyl}ethylidene)malononitrile (FDDNP), Pittsburgh compound B (PIB) and 18F-fluorodeoxyglucose (FDG). All of them have been successfully proved to differentially diagnose AD patients with mild cognitive impairment (MCI) and controls. This type of diagnosis is possible by targeting amyloid plaques, neurofilaments and detecting the decrease in cerebral glucose metabolism. In particular, PIB selectively binds cortical and striatal Aβ plaques, showing a positive correlation with AD diagnosis. However, PIB is also retained in normal individuals and further studies are needed to clarify if this could be a preclinical sign of AD.

Despite their sensitivity and reliability, these radiotracers generally show poor specificity for the diagnosis of dementia, because amyloid lesions and areas of metabolic reduction can also be present in other types of dementia. Moreover, it should also be noticed that plaques and tangles typical of AD patients are sometimes present in healthy elderly people (Reitz and Mayeux 2014). In vivo changes at molecular level can be detected by PET and single photon emission computed tomography (SPECT) also in PD patients. A significant reduction in vesicular monoamine transporter type 2, dopamine transporter and L-aromatic amino acid decarboxylase in the posterior putamen of affected patients can be detected by PET and SPECT with a specificity and sensitivity around 80-100% (123I of lupine (123I-FP-CIT;DaTSCAN TM). However, no imaging techniques are specific enough for a proper

differential diagnosis, thus making these methods not recommended for routine use in clinical practice (Pagano, Niccolini, and Politis 2016).

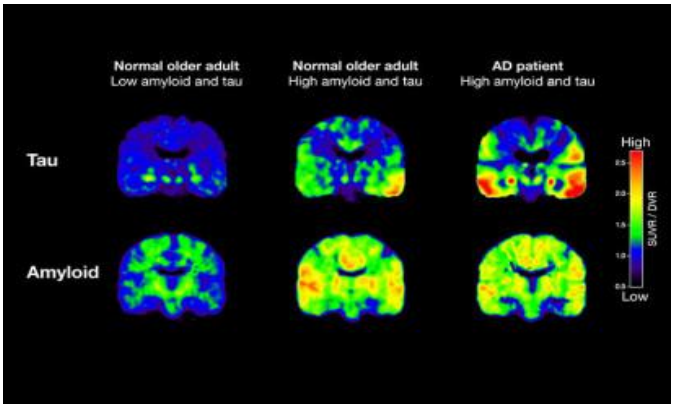

Figure 3. PET images of tau and Aβ lesions in normal elderly individuals and AD patients. Protein accumulation is evidenced by red and yellow signals both in the normal elderly adult (middle image) and in the AD patient (right image) (Images: https://www.alzheimer-riese.it, Michael Schöll).

The uniform glucose transport across the blood brain barrier in all the central nervous system is fundamental for a correct cerebral functionality. Therefore, a decreased glucose metabolism in specific brain areas is a sign of decreased cerebral activity linked to a loss of synapse number and activity. In particular hypometabolism of specific areas, that can be detected by FDG-PET, correlates with different AD stages. For example, a loss of ATP production in the medial temporal part of the brain is related to a diagnosis of late onset AD, while posterior lateral temporoparietal region hypometabolism well correlates with early disease onset. Therefore, glucose metabolism detection can be used as a marker of AD progression. Moreover, hypometabolism of the posterior cingulate-precuneus can also be found in MCI patients not yet classified as AD cases. In this sense, a deficit in glucose metabolism can be associated to a memory loss rather than an AD conversion (Nobili and Morbelli 2010).

Despite more selective imaging methods are continuously emerging, the current imaging techniques together with the clinical symptoms do not allow a specific NDD diagnosis. For these reasons, these tools are now only used as collateral methods to define a diagnosis that is still extremely imprecise.

Biomarkers and Their Biochemical Detection

Cerebrospinal fluid (CSF) and blood biomarker detection are also employed to support diagnosis of NNDs in combination with clinical symptoms, cognitive tests, and imaging data. Yet, biomarkers detection does not have a diagnostic value by itself in most NDDs.

Current Biomarkers in Alzheimer's Disease

AD pathophysiology can now be studied in vivo thanks to certain biomarkers in the CSF. Currently validated CSF biomarkers of AD are Aβ42, t-tau, and phosphorylated tau (p-tau) proteins. Aβ is the main component of AD plaques derived from amyloid precursor protein (APP). Tau is a microtubule-associated protein whose hyperphosphorylation leads to misfolded tau fibrils (tangles) that induce the formation and aggregation of neurofilaments. Although the majority of Aβ is produced and released in the brain extracellular space, a small fraction of the Aβ42 peptide can be found in the CSF in AD patients (10-15 ng/ml). Contrarily, the mechanism by which tau appears in CSF is unclear. Both CSF-Aβ42 and CSF-tau have been shown to be good markers for the presence of plaques deposition and neurodegeneration, especially when combined.

A large body of evidence shows that AD patients present decreased levels of Aβ42 and increased levels of t-tau and p-tau compared to healthy controls (Hampel et al. 2008; Mitchell

2009). Levels of Aβ42 in CSF have been shown to be reduced by 50% in AD patients compared to age matched controls likely due to a preferential deposition in the brain tissue during pathology (Motter et al. 1995). Moreover, only the combined detection of the three mentioned biomarkers (Aβ, tau, and p-tau) in the CSF significantly increases the diagnostic validity of the test for sporadic AD, with a combined sensitivity > 95% and a specificity >85% (Humpel and Hochstrasser 2011; Humpel 2011). These findings have led to the inclusion of these biomarkers in the revised diagnostic criteria for AD as supportive feature of the clinical diagnosis (McKhann et al. 2011). Unfortunately, the potential of the CSF biomarkers to discriminate AD from other forms of dementia is being questioned (Rivero-Santana et al. 2016). Recent studies comparing AD and other dementia suggest that specific variations of t-tau, p-tau, and Aβ42 concentrations in the CSF can help discriminating among different diseases. However, there is a substantial heterogeneity and inconsistency across studies (van Harten et al. 2011; McKhann et al. 2011). Moreover, CSF-Aβ42 levels do not appear to correlate with the progression and the severity of the disease, and ageing per se can alter CSF-tau concentration. If we consider that CSF tau levels are also detected in acute brain injury, that they remain stable through the course of AD, that they do not correlate with AD severity and change with age, tau per se shows a rather low diagnostic potential (Humpel 2011). While these evidences altogether show the pathological relevance of the known biomarkers, they also highlight the current impossibility to employ them as a stand-alone diagnostic reference in NDDs. Advancement in this field may be achieved through i) the identification of novel biomarkers more selective for specific AD and likely leading to the combined analysis of several biomarkers defining patient-specific signatures; ii) and /or by new methodologies allowing the detection of small amounts of pathological proteins at early disease stages and, of additional utmost importance, in easily accessible body fluids distinct from CSF, whose withdrawal requires a very invasive procedure that should be avoided.

The standard biochemical method today available to detect biomarkers in AD and other NDDs (see below) is the enzyme-linked immunosorbent assay (ELISA). ELISA detects the presence of a substance in a liquid/wet sample (such as CSF or blood serum) thanks to antibodies with specificity for a particular antigen. Despite the low detection limit of this technique (1 pg/ml), ELISA fails to reveal established AD biomarkers in blood samples or urine because of their very low amounts (Nimse et al. 2016). Furthermore, it does not allow the analysis of lowly and highly expressed proteins in the same assay, which increases the costs associated with diagnosis (Humpel 2011). Notably, the development of a multiplexed system to measure all reference biomarkers concurrently in a single assay is required (Humpel 2011). Due to these drawbacks, this methodology finds a limited application as a point of care detection tool for NDDs.

In attempt to establish more robust assays and new detection approaches, Savage et al. (2014), developed a modified ELISA assay to reveal Aβ-oligomers (which are more toxic than monomers). They used an antibody coupled to a sensitive, bead-based fluorescent platform able to detect the light emission of single photons. In this way, the authors reached a selectivity for oligomers more than 25,000-fold higher than that for monomers, with a significant 3 to 5-fold change increase in oligomer detection in the CSF of AD patients compared to age controls (63 AD vs 54 control patients) (Savage et al. 2014). However, specific biomarkers detection in CSF of AD patients is not yet validated as a method for everyday clinical diagnosis.

Moreover, to circumvent current ELISA-related flaws in sensitivity and selectivity especially for an early and discerning diagnosis in conditions of very low biomarker presence, a very recent study proposed a method to detect Aβ in blood samples thanks to immunoprecipitation approaches coupled with mass spectrometry (Nakamura et al. 2018). This method displayed an accuracy of 90% in predicting Aβ brain burden at an individual level, as confirmed with PET imaging. The possibility to use plasma biomarkers will surely bring relevant cost–benefit and scalability advantages over current techniques, potentially enabling broader clinical access and efficient population screening. However, while this study shows the potential predictive clinical utility of plasma biomarkers, the proposed methodology remains still far from clinical applications due to the need of further validation and standardization steps, and of the development of cost-effective automated assays.

Current Biomarkers in Parkinson's Disease

In PD studies inconsistent findings were obtained in the attempt to employ α-synuclein CSF concentration to distinguish PD from other NDDs (Farotti et al. 2017). For instance, two independent validated ELISA assays were unsuccessfully used to detect CSF total α-synuclein levels in order to distinguish PD patients from controls (Førland et al. 2018). These results support the possible inadequacy of α-synuclein as a single diagnostic and prognostic biomarkers and also highlight the need to improve the diagnostic technology. Unfortunately, the only reliable test is SPECT that can estimate the loss of dopamine terminals, and there are no unequivocal lab tests that can confirm clinical diagnosis features in PD, contributing to the relevant (10-20%) frequency of mis-diagnosis in this pathology.

Current Biomarkers in Multiple Sclerosis

Nowadays, the biomarkers used for the diagnosis and follow up of MS are Oligoclonal bands (OCBs) in the CSF, white matter lesions on MRI, and JC viral titer in serum blood. In particular, specific biomarkers would be extremely relevant to distinguish the four phenotypes of the disease: Clinical Isolated Syndrome (CIS), relapsing-remitting MS, primary and secondary progressive MS (Figure 4). However, as in AD, the number of biomarkers currently used in MS clinical practice is rather small and with a limited discriminative diagnostic value.

Since MS is a pathology that involves immune cells systemically, particularly relevant is the analysis of body fluids with ELISA methods to support the validity of the clinical diagnosis. The first biomarker included in the MS diagnostic criteria in 1983 was the CSF presence of IgG OCBs, bands of immunoglobulins that can be detected with protein electrophoresis. OCBs are used as indicators of MS since up to 95% of all patients with multiple sclerosis have permanently observable OCBs (Rivero-Santana et al. 2016). OCBs are still currently used also to predict the conversion from CIS to MS. Together with OCBs, an IgG index > 0.7 can confirm a suspicious demyelinating disease onset, supporting MS diagnosis (Comabella and Montalban 2014). However, a positive IgG index or OCBs presence cannot be considered a certain proof of disease. Indeed, around 10% of MS patients show a normal CSF composition, thus indicating the non-selectivity of this method. Moreover, CSF harvesting is an invasive procedure that is generally performed only once for each patient (Stangel et al. 2013).

ANTIBODY	CATEGORY	BODY FLUID	TARGET POPULATION	CLINICAL DECISION
Anti-natalizumab antibodies	Natalizumab-response biomarker for an adverse effect	Serum	Patients with MS given natalizumab	Yes, if persistently positive, treatment wirh natalizumab should be discontinued
Neutralising antibodies	Interferon-beta-response biomarker	Serum*, PBMCs	Patients with MS given interferon beta	Yes, if present a switch to a non-interferon-beta tratment should be considered
IgG oligoclonal bands	Diagnostic	CSF, serum	Patients suspected to have demyelinatind disease	No
IgG index	Diagnostic	CSF, serum	Patients suspected to have demyelinatind disease	No
Anti-aquaporin-4 antibodies	Diagnostic	Serum*, CSF	Patients with clinical and MRI features suggestive od neuromyelistis optica or neuromyelitis optica spectrum disorders	Yes, if present diagnosis should be separate from typical MS
Anti-JC virus antibodies	Natalizumab-response biomarker for an adverse effect	Serum*, plasme	Patients with MS eligible for natlizumab or those receiving natalizumab	Yes, test result allows stimation of the patient's risk for progressive multifocal leukoencephalopathy
Anti-JC antibodies	Fingolimod-response biomarker for an adverse effect	Serum*, plasme	Patients with MS eligible for fingolimod	Yes, seronegative patients should be vaccinated at least 1 month before start of fingolimod

Figure 4. Diagnostic biomarkers currently used in MS diagnosis.

The presence of specific types of antibodies (Abs) is also important in the clinic as supportive element for a differential diagnosis. This is the case of anti-acquaporin 4 Abs whose concentration in CSF/serum allows to specifically distinguish MS from Neuromyelitis Optica. Indeed, such autoimmune inflammatory disorder clinically overlaps with MS, and discrimination is essential as prognosis and treatment of the two pathologies are different (Lennon et al. 2004). Other classes of Abs, simply measured by a blood test, are monitored in relation to the employment of natalizumab, the most efficient drug for MS patients. Abs are produced by the patients against natalizumab after 3 months of treatment. The persistence of these antibodies in the serum has been associated with adverse effects and a reduced therapeutic efficacy, thus requiring the suspension of the treatment (Sørensen et al. 2011). Another class of Abs that are monitored in the serum/plasma are the so called anti-JC virus Abs, present in progressive multifocal leukoencephalopathy (PML) patients. PML is caused by the reactivation of the JC virus and emerged as a rare adverse event of natalizumab treatment. Therefore, the seropositivity for anti-JC Abs is now examined before and during the treatment with natalizumab, making this test a useful biomarker to assess the risk of PML. However, less than 1% of MS patients positive for JC Abs will develop PML, thus indicating the need for a more selective biomarker (Plavina et al. 2014).

Taken together, these evidences show how far we are from the employment of easily accessible and validated biomarkers for diagnosis of most NDDs. As a matter fact, nowadays NDD diagnosis-prognosis is still really imprecise, usually late and, at the moment, only the combination of imaging techniques and CSF protein concentration level is considered as a reliable diagnostic support, in order to exclude or confirm NDD diagnoses mainly based on clinical symptoms. Therefore, the current challenges to circumvent these limitations are many and comprise: i) a more selective detection of small amounts of validated known biomarkers, ii) the identification of novel selective biomarkers possibly defining disease and stage-specific signatures, iii) the employment of scalable samples obtained with minimally invasive approaches and adequate for screening, and iv) innovative, more selective and cost-effective detection methods.

Novel Biomarkers

Single protein biomarkers in the CSF, as well as multi-component biomarkers, and biomarkers based on gene expression constitute promising options for both early stage NDD detection and differential diagnosis (Lönneborg 2008).

As regards AD, an example of novel CSF biomarkers is provided by cargo proteins, such as chromogranin-B, α-synuclein, neuregulin-1, and nonamyloidogenic N-terminal fragment of APP (sAPPα) (Schaffer et al. 2015). In particular cargo protein can be monitored to indicate alteration in axonal transport, a common feature in neurodegeneration. In the CSF of three AD mouse model Fanara et al. (2012) used a heavy water (2H2O) pulse-deuterium labelling method that was able to detect delayed appearance and disappearance kinetics, advocated an anomalous axonal transport. Another CSF protein that could be employed for early diagnosis of AD is an astrocyte-derived protein, YKL-40 (Burman et al. 2016). This protein is an inflammatory biomarker highly up-regulated in patients with AD compared to normal subjects. Moreover, the amount of YKL-40 is statistically different in distinct clinically defined patient groups, thus it is possible to discern whether it is AD or cognitive impairment. On these bases, it has been proposed that a combined measurement of Aβ1-42 and YKL-40 may represent a new way to diagnose preclinical and early clinical stages of AD.

As highlighted above, a valuable methodology to facilitate AD diagnosis would be analysing biomarkers in body fluids like blood or urine. These samples are cheaper and are obtained with a better compliance of the patients. It is already known, for instance, that by analysing blood samples it is possible to detect a decrease of apoE4 levels, which can be associated with neuronal degeneration (Farrer et al. 1997; Lukens et al. 2009). Intriguingly, shortened telomere length in peripheral leukocytes has been proposed to indicate an individual's risk for developing AD, so in 2009 a study compared the telomere length in peripheral blood leukocyte and cerebellum samples of AD patients and controls. The results in the cerebellum were not significant at all (Patel et al. 2011), suggesting that reduced telomeres in leukocyte of AD patients may reflect changes in other regions of the brain.

Collecting urine from an AD patient could be an easy way to detect neuronal thread protein (NTP), in particular, AD7c-NTP, that correlates to neural dysfunction (Patel et al. 2011). In fact, high levels of that protein can be detected both in CSF and in urine of AD patients with a

monoclonal antibody. Unluckily, NTP can be detected more easily in patients who already have AD, thus not meeting the need of markers for early diagnosis (Lönneborg 2008). Unfortunately, all of these innovative methods still wait standardization and validation, even if they are considered to be very promising, especially when combined with other older better-known biomarkers.

In PD, in spite of massive efforts to discover new diagnostic methods, no specific biomarkers have been employed yet. The major issue is that, unfortunately, PD is a very heterogeneous disease that shares similar early symptoms with other NDDs. Nevertheless, during the last decade, the development of proteomics, metabolomics, and transcriptomics techniques hold great promises for the authentication of subtle alterations in protein, metabolites or RNA profiles in tissue and in body fluids (Caudle et al. 2010). All of these 'omics' techniques have been applied, ex vivo or in vivo, to brain tissue, CSF, blood and blood constituents to help, during preclinical stages, the identification of sensitive biomarkers that may help differentiating PD from other NDDs. In particular, an innovative way to diagnose PD could be constituted by transcriptomic alterations in pathways in the human substantia nigra pars compacta also detectable in blood of PD patients, but also novel genes and proteins like mortaline, GATA-2, and ST13. In addition, a differential metabolic based profile was observed in patients with idiopathic PD, PD with G2019S LRRK2 mutation, asymptomatic G2019S LRRK2 and normal controls (Caudle et al. 2010). Furthermore, proteomics helped researches to characterize the human midbrain and the composition of the CSF, and these studies will be used to recognize altered proteins and pathways in body fluids of PD subjects. Indeed, in 2009 Johansen et al. discriminate plasma metabolic profile of PD and LRRK2 PD patients compared to controls, and they were able to discern patients from normal subjects. They observed a peculiar decrease in uric acid level in both PD and LRRK2 PD patients plasma and a decreased level of hypoxanthine and purine pathway in PD patients plasma (Johansen et al. 2009).

Omics techniques have been also exploited extensively to find and validate MS biomarkers, together with studies on micro RNAs (Vistbakka et al. 2017; Regev et al. 2016). However, these studies have not yet reached clinical applications mainly because of inconsistency of results. Other potential markers could be neurofilaments (NFL) and glial fibrillary acidic protein (GFAP). NFL levels in CSF of MS patients are elevated (Kuhle et al. 2015) and can be easily determined by ELISA. Recently Kuhle and collaborators (2016) have further demonstrated that serum NFL levels could be a potential biomarker of on-going disease progression, but these evidences need to be reconfirmed in larger cohorts of patients. Another neuronal and glial cell damage biomarker that shows high level in MS patients is GFAP. In particular, it correlates to reduced ambulation and severe disability (Petzold et al. 2002). On the other hand, many other molecules that appeared very promising in single studies with quite a small cohort of patients (serum anti-MBP and anti-myelin oligodendrocyte glycoprotein antibodies in patients with CIS, cleaved cystatin C in the CSF, CSF soluble Nogo-A, serum soluble HLA-G, and serum IL17F as a response biomarker for interferon-beta treatment) turned out to be useless for the clinical practice (Alsahebfosoul et al. 2000; Lindsey, Crawford, and Hatfield 2008; Kuhle et al. 2007). In fact, unfortunately, the current medical tools are still not enough sensitive or specific for the prediction and the monitorization of neurodegenerative diseases and treatment effects.

Despite their presence in clinical practice, the number of biomarkers currently used in NDDs diagnosis and prognosis is still very low compared to the great number of validated or exploratory molecules now under investigations.

Graphene Technology

The need to overcome the current limitation in diagnostic approaches through the development of diagnostic tools with enhanced selectivity and sensitivity, as well as the pressing demand for new, more selective biomarkers, has recently attracted attention on graphene and its derivatives, as new materials for biosensing applications (Terse-Thakoor, Badhulika, and Mulchandani 2017). Graphene is a two-dimensional (2D) material, composed of a monolayer of carbon atoms, and considered as the world's first 2D nanomaterial (Figure 5), originally isolated by Andre Geim and Kostia Novoselov, two researchers of the University of Manchester who won the Nobel prize in physics for the discovery (Geim and Novoselov 2007). This single atomic layer of carbon is transparent, strong and at the same time flexible. It is, in addition, an electricity conductor (Pumera et al. 2010; Goenka, Sant, and Sant 2014; Lawal 2015). It possesses unique physicochemical properties due to its large surface to volume ratio, excellent thermal and electrical conductivity, biocompatibility, as well as broad electrochemical potential

(Terse-Thakoor, Badhulika, and Mulchandani 2017). These features, together with the presence of reactive edges that facilitate functionalization with biorecognition elements, have made graphene a popular material for the development of electrochemical or optical sensors. These sensors showed enhanced sensitivity and specificity thanks to the integration of metals, metal oxides and quantum dots (Gao and Duan 2015; Favero et al. 2015; Song et al. 2016) for both ex situ and in situ applications. A further appealing characteristic of this material is the low cost, that significantly adds to the unique selling point of graphene, and propels research on this material in biomedical applications (Defteralı et al. 2016).

The development of graphene-based biosensors and electrochemical sensors helping in detecting a wide array of analyte and biological entities such as DNA, proteins, and pathogens, has indeed started in several medicine fields (Justino et al. 2017) (Figure 6). The large surface area of graphene can enhance the surface loading of desired biomolecules, and excellent conductivity and small band gap can be beneficial for conducting electrons between biomolecules and the electrode surface. Graphene biosensors are therefore under development for the detection of a range of analytes like glucose, neurotransmitters, cholesterol, hemoglobin and more. Moreover, graphene also has a significant potential for electrochemical biosensors based on direct electron transfer between the enzyme and the electrode surface. For instance, in cancer research, microfluidic chips with a graphene sensor have been developed to sense and isolate cancer cells with capture yields and detection sensitivities that were much higher than those reported for conventional approaches (Reina et al. 2017). Graphene materials have also successfully been employed as electrochemical sensors in antibody-antigen based platforms for the detection of cancer proteins. In these applications, the intrinsic sensitivity and specificity of the antibody/antigen interaction was implemented by the exceptional transduction sensitivity of graphene. In these applications, apart from the improved electron transfer properties in the electrochemical measurements, graphene-based electrodes provided also the advantage of detection of multiple antigens (Reina et al. 2017). Notably, in cancer studies the increased sensitivity provided by the employment of graphene suggests that graphene biosensors in a near future may represent an efficient way to detect low quantities of cancer biomarkers, allowing cancer diagnosis at early stages (Ghanbarzadeh and Hamishehkar 2017; Balaji and Zhang 2017; Cruz et al. 2016; Pasinszki et al. 2017).

On these bases, graphene-based technology has been investigated also in NDD for both therapeutic and diagnostic (biomarkers detection) applications. The low cost and potential increased sensitivity and selectivity conferred by graphene-based sensors may allow both the early detection of low amounts of pathological biomarkers and the employment of easy-to-obtain body fluids (distinct from CSF), where relevant molecules are present at very low concentration, which makes them very difficult to detect with conventional methodologies. In particular, we report studies that prospect graphene-based applications for the cure and the diagnosis of AD, PD and MS.

Graphene-Based Materials

The great majority of the applications of graphene-derived materials employ Graphene Quantum Dots (GQDs) and Graphene Oxide (GO). In particular, GQDs are a single-or few-layer graphene with a size less than 100 nm, that are greatly biocompatible, low cytotoxic and have photoluminescence and hydrophobic properties. Moreover, GO (Figure 5) is an oxidized form of graphene, with high-density oxygen functional groups (like hydroxyl, epoxy and carboxyl group), and it is dispersible in water (and other solvents) (Yu et al. 2016).

Researchers' close attention to the world of biomaterials led to the rise of a new class of graphene-like 2D materials (2DMats) (Bollella et al. 2017). This emerging class of materials (including boron nitride (BN), graphite-carbon nitride (g-C3N4), transition metal dichalcogenide (TMDs), transition metal oxide and graphane) is considered unique for its properties, which allow applications in energy conversion, catalysis, biosensing and in the biomedical field.

The core structure of BN nanosheets is based on alternate boron and nitrogen atoms acting as an insulator, covered by a honeycomb lattice structure (Lin and Connell 2012). Graphite-carbon nitride is, instead, a polymeric material composed by C, N and H atoms interconnected via tris-triazine-based motifs and it is considered a semiconductor (Zhu et al. 2014).

TMDs, differently from graphene, do not consist of a single layer of atoms. They are made of a layer of transition metals such as molybdenum, tungsten or niobium, included in two layers of chalcogen atoms. This sandwich structure is linked by weak van der Waals bonds, whereas the atoms are held together by covalent bonds (Lv et al. 2015). TMDs and graphene share

some properties, as they are thin and strong. However, unlike graphene, most of TMDs are semiconductors although some of them are semimetals or metals.

Transition metal oxides are functional materials, which have many applications because of their different properties, so that there is a new interest on the development of new structures, such as nanotubes and nanofibers (Sun et al. 2015). Finally, graphane is a hydrogenated form of graphene, and it is characterized by a reversible hydrogenation that allows controlling its conductive properties, from insulator to conductor (Zhou et al. 2014). This property leads to its use for high sensitive nanosensors.

If 2DMats are still under intense investigation and are far from their employment, GDQs and GO technologies are largely applied with encouraging results in the research field of NDDs, in particular for (i) AD, (ii) PD and (iii) MS.

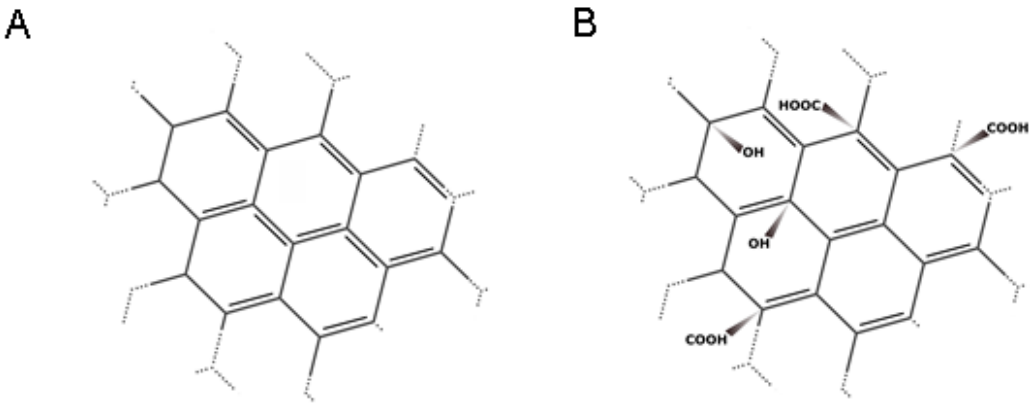

Figure 5. Structure of graphene. A) Graphene, B) Graphene Oxide.

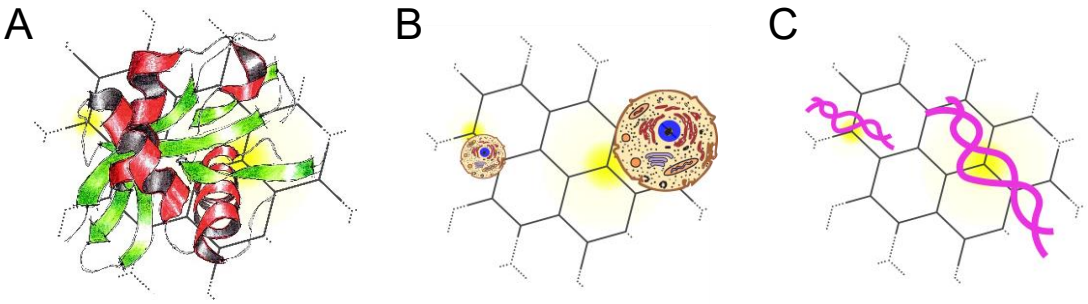

Figure 6. Schematic representation of possible applications of graphene. Graphene for protein (A), cell (B) and nucleic acid (C) detection.

Graphene-Based Applications in Alzheimer's Disease

AD is characterized by the aggregation of Aβ peptides and the central motif of Aβ1-42 is hydrophobic. Because some studies proved that modifications of hydrophobic regions can promote the disassembly of Aβ fibrils (R. Liu et al. 2004; Tao L. Lowe et al. 2001), GQDs, for their hydrophobic properties, have been widely studied. In particular, Liu Y et al. (2015) provided a proof-of-concept of the ability of GQDs to inhibit Aβ1-42 peptides aggregation, employing a thioflavin T (ThT) fluorescence assay to monitor fibrils formation; rescue Aβ induced cytotoxicity treating PC12 cells with peptides alone or in the presence of GQDs; GQDs are also able to increase cell survival of about 80% (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

– MTT – assay). As far as we know, this is the first study that prove the ability of GQDs to inhibit Aβ1-42 aggregation and that promotes the development of therapeutic drugs for AD. Moreover, GQDs can also be used as conjugated forms. Songhua Xiao et al. (2016) designed a new nanomaterial called graphene quantum dots conjugated neuroprotective peptide glycine-proline-glutamate (GQDG) that has been administered intravenously to a mouse model of AD. The Morris Water Maze test demonstrated a better cognitive ability related to the control group, which indicates that treatment with GQDG could improve learning and memory capability. For what concerns immunohistochemical analysis, researchers showed that GQDG lead to a decrease of Aβ plaques, to a reduction of microglial activation, an increase of dendritic spines, that correlates to the improve in learning and memory capability and an increase in newly generated cells. Based on these encouraging findings, GQDs could be used for the development of therapeutic drugs for AD.

Researchers (Huang et al. 2017) proposed that GQDs can be used as a detector of monomeric amyloid peptides. Detection of the concentration of amyloid monomers is important in the diagnosis of AD. To investigate protein amyloidogeneis, Thioflavin T (ThT) is the prevalent probe for monitoring and visualizing Aβ fibrils (Biancalana and Koide 2010). GQDs, thanks to the fluorescence of graphene, can actually be used as a detection probe comparably to ThT. Furthermore, unlike ThT they do not need a co-incubation with the fibrillogenesis system that might bring interference into the system.

For what concerns GO, in an interesting study Demeritte T. et al (2015) developed an antibody-conjugated platform to detect AD biomarkers. They employed a magnetic core-plasmonic shell nanoparticle attached to hybrid graphene oxide that capture with a sensitivity of more than 98% AD biomarkers (Aβ and tau protein) from whole blood sample. Such high sensitivity is due to the strong plasmon-coupling which generates huge amplified electromagnetic fields, a feature exploited by surface enhanced Raman spectroscopy (SERS) (X. Xu et al., 2013). Therefore, despite further investigations are necessary to optimize graphene-derived materials in AD therapy and biomarker detection, the results obtained until now make graphene one of the best candidate for biosensor engineering.

Graphene-based Applications in Parkinson's Disease

In analogy with therapeutic applications in AD, GQDs were used not only to inhibit α-synucleinfibrillization but also to disaggregate mature fibrils (Kim et al. 2017) (Figure 7). In addition, in vivo administration of GQDs protects against α-synuclein-dependent loss of dopamine neurons and behavioural deficits through the penetration of the blood-brain barrier (BBB).

We know that one of the major properties of graphene is its metallic conductance and its large accessible surface area. For these reasons, graphene has been studied for impedimetric biosensing. GO is particularly interesting because it possesses hydroxyl, epoxy, and carboxyl functional groups that can be functionalized with different reagents.

On the side of biosensor development, Xu et al. (2015) provided a proof for the use of GO in the detection and quantification of serum-based α-synuclein autoantibodies. In particular, they reported that the use of cysteamine-graphene oxide modified gold arrays in the quantification of Parkinson's-relevant autoantibodies could be a good platform for diagnosis of preclinical PD. Another study, thanks to the nanoimprint lithography technique, developed a GO-based microelectrode array on a flexible platform in order to selectively detect dopamine and H2O2 at remarkable low concentrations (Reina et al. 2017). In this case, the authors successfully combined the intrinsic advantages of graphene with the device miniaturization into a microelectrode without altering the sensing properties. These two examples demonstrate the promising applicability of graphene-based materials also to detect PD biomarkers, thereby opening promising perspectives also for other NDDs.

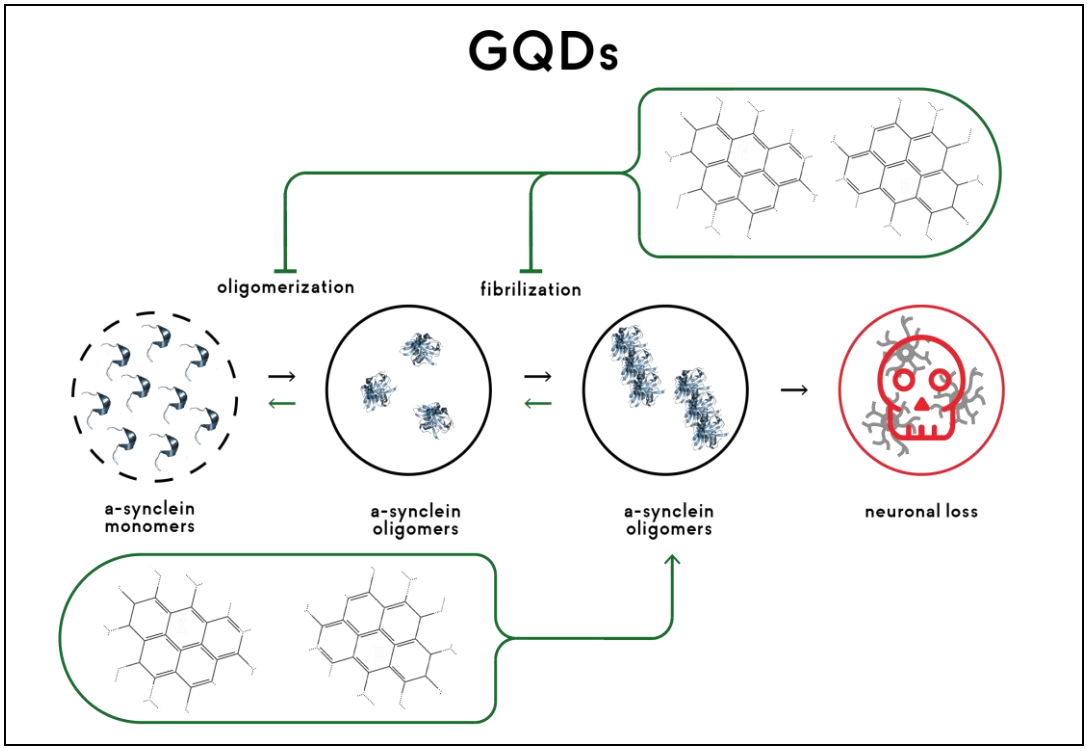

Figure 7. Schematic representation of GQDs ability to disaggregate α-synuclein fibrils, and to prevent their formation.

Graphene-Based Applications in Multiple Sclerosis

GO technology has also been applied in MS, with the same rationale of PD. In MS, clinical examination consists in magnetic resonance imaging (MRI) and CSF biochemistry measurements, which includes Immunoglobulin G (IgG), Myelin Basic Protein and tau quantifications. However, these methods are expensive and time consuming and especially not so efficient for early diagnosis.

Derkus B. et al. (2017) developed a nanoimmunosensor in which a screen-printed carbon electrode (SPCE) is modified with GO and amine functionalized 1st generation trimethylolpropanetris[poly(propyleneglycol)] (pPG) and then is conjugated with Myelin Basic Protein and tau antibodies. These nanoimmunosensors can detect and successfully measure two MS biomarkers, tau, and MBP, in CSF and serum. Moreover, nanosensors result to be fairly close to the commercially available ELISA in terms of sensitivity and allow the quantification of two biomarkers simultaneously. Even if these data are only preliminary, this study provides a proof-of-concept for the clinical use of graphene-based nanoimmunosensors in MS.

To sum up, there are several studies on the development graphene-based strategies that could be applied for NDDs. In particular, besides their potential therapeutic applications, GO platforms and GQDs can support the development of cost-effective sensors with potential increased sensitivity and selectivity toward multiple biomarkers, allowing both the early detection of low amounts of pathological biomarkers, and the employment of easy-to-obtain body fluids (distinct from CSF), where relevant molecules may be present at concentrations undetectable with conventional methodologies.

Conclusions and Future Perspective

There is an urgent need for selective and effective treatments to face the lack of efficient drugs to cure NDDs. Thus, nowadays research aims at ameliorating biomedical diagnostic tools and optimizing biomarkers detection, because early diagnosis may be the key for a successful prevention and cure. Although novel molecules are emerging as encouraging biomarkers, established targets could still reveal an enhanced diagnostic power, if detected in low amounts at early stages of disease and/or in body fluids easily accessible thanks to the development of

innovative biomedical sensors, with high specificity and sensitivity. In this frame, because of its physical properties and affordability, graphene has started to be employed to build ultrasensitive and potentially miniaturized biosensors for early detection and differential diagnosis of NDDs.

It can be further envisaged that, through graphene-based inexpensive sensor technology applied on easily accessible body fluids (e.g. blood, urine), multiple types of biological targets (e.g. proteins, transcripts, metabolites) will be detected in large-scale studies to provide complex multi-modal inputs to deep neural networks, which are efficient algorithms designed to recognize patterns. Extracted features can be fed to deep learning algorithms leading to the identification of new and more selective biomarkers for NDD correct diagnosis and prognosis, in a fashion similar to what is nowadays happening in aging, cancer and in pharmaceutical research, where growing datasets are mined for biomarker development and the identification of predictive trajectories. Identified biomarkers may be further implemented in a routine risk-stratification protocol prospectively valuable also to develop an economic model defining the most cost-effective screening strategies exploitable for health plans.

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References

Alsahebfosoul, Fereshteh, Ahmad Zavaran-Hosseini, Rasoul Salehi, Masood Etemadifar, Nafiseh Esmaeil, and Azam Jamshidian. 2000. *Iranian Journal of Allergy, Asthma and Immunology*. *Iranian Journal of Allergy, Asthma and Immunology*. Vol. 14. TUMS Publications.

Balaji, Aditya, and Jin Zhang. 2017. “Electrochemical and Optical Biosensors for Early-Stage Cancer Diagnosis by Using Graphene and Graphene Oxide.” *Cancer Nanotechnology* 8 (1): 10. doi:10.1186/s12645-017-0035-z.

Biancalana, Matthew, and Shohei Koide. 2010. “Molecular Mechanism of Thioflavin-T Binding to Amyloid Fibrils.” *Biochimica et Biophysica Acta* 1804 (7). NIH Public Access: 1405–12. doi:10.1016/j.bbapap.2010.04.001.

Bollella, Paolo, Giovanni Fusco, Cristina Tortolini, Gabriella Sanzò, Gabriele Favero, Lo Gorton, and Riccarda Antiochia. 2017. “Beyond Graphene: Electrochemical Sensors and Biosensors for Biomarkers Detection.” *Biosensors and Bioelectronics* 89 (March). Elsevier: 152–66. doi:10.1016/J.BIOS.2016.03.068.

Burman, Joachim, Raili Raininko, Kaj Blennow, Henrik Zetterberg, Markus Axelsson, and Clas Malmeström. 2016. “YKL-40 Is a CSF Biomarker of Intrathecal Inflammation in Secondary Progressive Multiple Sclerosis.” *Journal of Neuroimmunology* 292 (March): 52–57. doi:10.1016/j.jneuroim.2016.01.013.

Caudle, W Michael, Theo K Bammler, Yvonne Lin, Sheng Pan, and Jing Zhang. 2010. “Using ‘omics’ to Define Pathogenesis and Biomarkers of Parkinson’s Disease.” *Expert Review of Neurotherapeutics* 10 (6): 925–42. doi:10.1586/ern.10.54.

Celone, K. A., V. D. Calhoun, B. C. Dickerson, A. Atri, E. F. Chua, S. L. Miller, K. DePeau, et al. 2006. “Alterations in Memory Networks in Mild Cognitive Impairment and Alzheimer’s Disease: An Independent Component Analysis.” *Journal of Neuroscience* 26 (40): 10222–31. doi:10.1523/JNEUROSCI.2250-06.2006.

Comabella, Manuel, and Xavier Montalban. 2014. “Body Fluid Biomarkers in Multiple Sclerosis.” *The Lancet Neurology* 13 (1): 113–26. doi:10.1016/S1474-4422(13)70233-3.

Cruz, Sandra M A, André F Girão, Gil Gonçalves, and Paula A A P Marques. 2016. “Graphene: The Missing Piece for Cancer Diagnosis?” *Sensors (Basel, Switzerland)* 16 (1). Multidisciplinary Digital Publishing Institute (MDPI). doi:10.3390/s16010137.

Defteralı, Çağla, Raquel Verdejo, Shahid Majeed, Adriana Boschetti-de-Fierro, Héctor R. Méndez-Gómez, Eva Díaz-Guerra, Daniel Fierro, et al. 2016. “In Vitro Evaluation of Biocompatibility of Uncoated Thermally Reduced Graphene and Carbon Nanotube-Loaded PVDF Membranes with Adult Neural Stem Cell-Derived Neurons and Glia.” *Frontiers in Bioengineering and Biotechnology* 4 (December): 94. doi:10.3389/fbioe.2016.00094.

Demeritte, Teresa, Bhanu Priya Viraka Nellore, Rajashekhar Kanchanapally, Sudarson Sekhar Sinha, Avijit Pramanik, Suhash Reddy Chavva, and Paresh Chandra Ray. 2015. “Hybrid Graphene Oxide Based Plasmonic-Magnetic Multifunctional Nanoplatform for Selective Separation and Label-Free Identification of Alzheimer’s Disease Biomarkers.” *ACS Applied Materials & Interfaces* 7 (24). American Chemical Society: 13693–700. doi:10.1021/acsami.5b03619.

Derkus, Burak, Pinar Acar Bozkurt, Metin Tulu, Kaan C Emregul, Canan Yucesan, and Emel Emregul. 2017. “Simultaneous Quantification of Myelin Basic Protein and Tau Proteins in Cerebrospinal Fluid and Serum of Multiple Sclerosis Patients Using Nanoimmunosensor.” *Biosensors & Bioelectronics* 89 (Pt 2): 781–88. doi:10.1016/j.bios.2016.10.019.

Dickerson, B. C., D. H. Salat, D. N. Greve, E. F. Chua, E. Rand-Giovannetti, D. M. Rentz, L. Bertram, et al. 2005. “Increased Hippocampal Activation in Mild Cognitive Impairment Compared to Normal Aging and AD.” *Neurology* 65 (3): 404–11. doi:10.1212/01.wnl.0000171450.97464.49.

Fanara, Patrizia, Po-Yin A. Wong, Kristofor H. Husted, Shanshan Liu, Victoria M. Liu, Lori A. Kohlstaedt, Timothy Riiff, et al. 2012. “Cerebrospinal Fluid–based Kinetic Biomarkers of Axonal Transport in Monitoring Neurodegeneration.” *Journal of Clinical Investigation* 122 (9): 3159–69. doi:10.1172/JCI64575.

Farotti, Lucia, Silvia Paciotti, Anna Tasegian, Paolo Eusebi, and Lucilla Parnetti. 2017. “Discovery, Validation and Optimization of Cerebrospinal Fluid Biomarkers for Use in Parkinson’s Disease.” *Expert Review of Molecular Diagnostics* 17 (8). Taylor & Francis: 771–80. doi:10.1080/14737159.2017.1341312.

Farrer, L A, L A Cupples, J L Haines, B Hyman, W A Kukull, R Mayeux, R H Myers, M A Pericak-Vance, N Risch, and C M van Duijn. 1997. “Effects of Age, Sex, and Ethnicity on the Association between Apolipoprotein E Genotype and Alzheimer Disease. A Meta-Analysis. APOE and Alzheimer Disease Meta Analysis Consortium.” *JAMA* 278 (16): 1349–56. http://www.ncbi.nlm.nih.gov/pubmed/9343467.

Favero, Gabriele, Giovanni Fusco, Franco Mazzei, Federico Tasca, and Riccarda Antiochia. 2015. “Electrochemical Characterization of Graphene and MWCNT Screen-Printed Electrodes Modified with AuNPs for Laccase Biosensor Development.” *Nanomaterials* 5 (4): 1995–2006. doi:10.3390/nano5041995.

Førland, Marthe Gurine, Annika Öhrfelt, Ingvild Dalen, Ole-Bjørn Tysnes, Kaj Blennow, Henrik Zetterberg, Kenn Freddy Pedersen, Guido Alves, and Johannes Lange. 2018. “Evolution of Cerebrospinal Fluid Total α-Synuclein in Parkinson’s Disease.” *Parkinsonism & Related Disorders*, January. doi:10.1016/j.parkreldis.2018.01.018.

Gao, Hongcai, and Hongwei Duan. 2015. “2D and 3D Graphene Materials: Preparation and Bioelectrochemical Applications.” *Biosensors & Bioelectronics* 65 (March): 404–19. doi:10.1016/j.bios.2014.10.067.

Geim, A. K., and K. S. Novoselov. 2007. “The Rise of Graphene.” *Nature Materials* 6 (3): 183–91. doi:10.1038/nmat1849.

Ghanbarzadeh, S., and H. Hamishehkar. 2017. “Application of Graphene and Its Derivatives in Cancer Diagnosis and Treatment.” *Drug Research* 67 (12). © Georg Thieme Verlag KG: 681–87. doi:10.1055/s-0042-115638.

Ghasemi, Nazem, Shahnaz Razavi, and Elham Nikzad. 2017. “Multiple Sclerosis: Pathogenesis, Symptoms, Diagnoses and Cell-Based Therapy.” *Cell Journal* 19 (1): 1–10.

Goenka, Sumit, Vinayak Sant, and Shilpa Sant. 2014. “Graphene-Based Nanomaterials for Drug Delivery and Tissue Engineering.” *Journal of Controlled Release* 173 (January): 75–88. doi:10.1016/j.jconrel.2013.10.017.

Golby, A., Gerald Silverberg, Elizabeth Race, Susan Gabrieli, James O’Shea, Kyle Knierim, Glenn Stebbins, and John Gabrieli. 2005. “Memory Encoding in Alzheimer’s Disease: An fMRI Study of Explicit and Implicit Memory.” *Brain* 128 (4): 773–87. doi:10.1093/brain/awh400.

Hampel, Harald, Katharina Bürger, Stefan J. Teipel, Arun L.W. Bokde, Henrik Zetterberg, and Kaj Blennow. 2008. “Core Candidate Neurochemical and Imaging Biomarkers of Alzheimer’s Disease.” *Alzheimer’s & Dementia* 4 (1): 38–48. doi:10.1016/j.jalz.2007.08.006.

Harten, Argonde C. van, Maartje I. Kester, Pieter-Jelle Visser, Marinus A. Blankenstein, Yolande A.L. Pijnenburg, Wiesje M. van der Flier, and Philip Scheltens. 2011. “Tau and P-Tau as CSF Biomarkers in Dementia: A Meta-Analysis.” *Clinical Chemistry and Laboratory Medicine* 49 (3). Walter de Gruyter: 353–66. doi:10.1515/CCLM.2011.086.

Huang, Huan, Ping Li, Min Zhang, Yue Yu, Yuhao Huang, Huaiyu Gu, Chen Wang, and Yanlian Yang. 2017. “Graphene Quantum Dots for Detecting Monomeric Amyloid Peptides.” *Nanoscale* 9 (16). Royal Society of Chemistry: 5044–48. doi:10.1039/C6NR10017A.

Humpel, Christian. 2011. “Identifying and Validating Biomarkers for Alzheimer’s Disease.” *Trends in Biotechnology* 29 (1). Elsevier: 26–32. doi:10.1016/j.tibtech.2010.09.007.

Humpel, Christian, and Tanja Hochstrasser. 2011. “Cerebrospinal Fluid and Blood Biomarkers in Alzheimer’s Disease.” *World Journal of Psychiatry* 1 (1). Baishideng Publishing Group Inc: 8–18. doi:10.5498/wjp.v1.i1.8.

Johansen, Krisztina K., Lei Wang, Jan O. Aasly, Linda R. White, Wayne R. Matson, Claire Henchcliffe, M. Flint Beal, and Mikhail Bogdanov. 2009. “Metabolomic Profiling in LRRK2-Related Parkinson’s Disease.” Edited by Mark R. Cookson. *PLoS ONE* 4 (10). Public Library of Science: e7551. doi:10.1371/journal.pone.0007551.

Justino, Celine I.L., Ana R. Gomes, Ana C. Freitas, Armando C. Duarte, and Teresa A.P. Rocha-Santos. 2017. “Graphene Based Sensors and Biosensors.” *TrAC Trends in Analytical Chemistry* 91 (June). Elsevier: 53–66. doi:10.1016/J.TRAC.2017.04.003.

Karas, Giorgos, Philip Scheltens, Serge Rombouts, Ronald van Schijndel, Martin Klein, Bethany Jones, Wiesje van der Flier, Hugo Vrenken, and Frederik Barkhof. 2007. “Precuneus Atrophy in Early-Onset Alzheimer’s Disease: A Morphometric Structural MRI Study.” *Neuroradiology* 49 (12): 967–76. doi:10.1007/s00234-007-0269-2.

Kim, Donghoon, Je Min Yoo, Heehong Hwang, Su Hyun Lee, Seung Pil Yun, Myung Jin Park, Seulah Choi, et al. 2017. “Graphene Quantum Dots Prevent {\alpha}-Synuclein Transmission in Parkinson’s Disease,” October. http://arxiv.org/abs/1710.07213.

Kivistö, Julia, Hilkka Soininen, and Maija Pihlajamaki. 2014. “Functional MRI in Alzheimer’s Disease.” In *Advanced Brain Neuroimaging Topics in Health and Disease - Methods and Applications*. InTech. doi:10.5772/58264.

Kuhle, J., G. Disanto, J. Lorscheider, T. Stites, Y. Chen, F. Dahlke, G. Francis, et al. 2015. “Fingolimod and CSF Neurofilament Light Chain Levels in Relapsing-Remitting Multiple Sclerosis.” *Neurology* 84 (16): 1639–43. doi:10.1212/WNL.0000000000001491.

Kuhle, Jens, Christian Barro, Giulio Disanto, Amandine Mathias, Charlotte Soneson, Guillaume Bonnier, Özguer Yaldizli, et al. 2016. “Serum Neurofilament Light Chain in Early Relapsing Remitting MS Is Increased and Correlates with CSF Levels and with MRI Measures of Disease Severity.” *Multiple Sclerosis Journal* 22 (12): 1550–59. doi:10.1177/1352458515623365.

Kuhle, Jens, Christoph Pohl, Matthias Mehling, Gilles Edan, Mark S. Freedman, Hans-Peter Hartung, Chris H. Polman, et al. 2007. “Lack of Association between Antimyelin Antibodies and Progression to Multiple Sclerosis.” *New England Journal of Medicine* 356 (4): 371–78. doi:10.1056/NEJMoa063602.

Lawal, Abdulazeez T. 2015. “Synthesis and Utilisation of Graphene for Fabrication of Electrochemical Sensors.” *Talanta* 131 (January): 424–43. doi:10.1016/j.talanta.2014.07.019.

Lennon, Vanda A, Dean M Wingerchuk, Thomas J Kryzer, Sean J Pittock, Claudia F Lucchinetti, Kazuo Fujihara, Ichiro Nakashima, and Brian G Weinshenker. 2004. “A Serum Autoantibody Marker of Neuromyelitis Optica: Distinction from Multiple Sclerosis.” *The Lancet* 364 (9451): 2106–12. doi:10.1016/S0140-6736(04)17551-X.

Lin, Yi, and John W. Connell. 2012. “Advances in 2D Boron Nitride Nanostructures: Nanosheets, Nanoribbons, Nanomeshes, and Hybrids with Graphene.” *Nanoscale* 4 (22). The Royal Society of Chemistry: 6908. doi:10.1039/c2nr32201c.

Lindsey, J. W., M. P. Crawford, and L. M. Hatfield. 2008. “Soluble Nogo-A in CSF Is Not a Useful Biomarker for Multiple Sclerosis.” *Neurology* 71 (1): 35–37. doi:10.1212/01.wnl.0000314831.08803.21.

Liu, Ruitian, Chad McAllister, Yuri Lyubchenko, and Michael R. Sierks. 2004. “Residues 17-20 and 30-35 of Beta-Amyloid Play Critical Roles in Aggregation.” *Journal of Neuroscience Research* 75 (2). Wiley Subscription Services, Inc., A Wiley Company: 162–71. doi:10.1002/jnr.10859.

Liu, Yibiao, Li-Ping Xu, Wenhao Dai, Haifeng Dong, Yongqiang Wen, and Xueji Zhang. 2015. “Graphene Quantum Dots for the Inhibition of β Amyloid Aggregation.” *Nanoscale* 7 (45). Royal Society of Chemistry: 19060–65. doi:10.1039/C5NR06282A.

Lönneborg, Anders. 2008. “Biomarkers for Alzheimer Disease in Cerebrospinal Fluid, Urine, and Blood.” *Molecular Diagnosis & Therapy* 12 (5): 307–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18803429.

Lukens, J. Nicholas, Vivianna Van Deerlin, Christopher M. Clark, Sharon X. Xie, and F. Brad Johnson. 2009. “Comparisons of Telomere Lengths in Peripheral Blood and Cerebellum in Alzheimer’s Disease.” *Alzheimer’s & Dementia* 5 (6). Elsevier: 463–69. doi:10.1016/J.JALZ.2009.05.666.

Lv, Ruitao, Joshua A. Robinson, Raymond E. Schaak, Du Sun, Yifan Sun, Thomas E. Mallouk, and Mauricio Terrones. 2015. "Transition Metal Dichalcogenides and Beyond: Synthesis, Properties, and Applications of Single- and Few-Layer Nanosheets." *Accounts of Chemical Research* 48 (1). American Chemical Society: 56–64. doi:10.1021/ar5002846.

Martin Prince, Authors, Anders Wimo, Maëlenn Guerchet, Miss Gemma-Claire Ali, Yu-Tzu Wu, Matthew Prina, Kit Yee Chan, and Zhiyu Xia. 2015. "World Alzheimer Report 2015 The Global Impact of Dementia An AnAlysIs of prevAlence, IncIDence, cosT AnD TrenDs." https://www.alz.co.uk/research/WorldAlzheimerReport2015.pdf.

McKhann, Guy M., David S. Knopman, Howard Chertkow, Bradley T. Hyman, Clifford R. Jack, Claudia H. Kawas, William E. Klunk, et al. 2011. "The Diagnosis of Dementia due to Alzheimer's Disease: Recommendations from the National Institute on Aging-Alzheimer's Association Workgroups on Diagnostic Guidelines for Alzheimer's Disease." *Alzheimer's & Dementia* 7 (3): 263–69. doi:10.1016/j.jalz.2011.03.005.

Mitchell, A J. 2009. "CSF Phosphorylated Tau in the Diagnosis and Prognosis of Mild Cognitive Impairment and Alzheimer's Disease: A Meta-Analysis of 51 Studies." *Journal of Neurology, Neurosurgery & Psychiatry* 80 (9): 966–75. doi:10.1136/jnnp.2008.167791.

Motter, R., C. Vigo-Pelfrey, D. Kholodenko, R. Barbour, K. Johnson-Wood, D. Galasko, L. Chang, et al. 1995. "Reduction of ?-Amyloid peptide42 in the Cerebrospinal Fluid of Patients with Alzheimer's Disease." *Annals of Neurology* 38 (4). Wiley Subscription Services, Inc., A Wiley Company: 643–48. doi:10.1002/ana.410380413.

Nakamura, Akinori, Naoki Kaneko, Victor L. Villemagne, Takashi Kato, James Doecke, Vincent Doré, Chris Fowler, et al. 2018. "High Performance Plasma Amyloid-β Biomarkers for Alzheimer's Disease." *Nature*, January. Nature Publishing Group. doi:10.1038/nature25456.

Nimse, Satish Balasaheb, Mukesh Digambar Sonawane, Keum-Soo Song, and Taisun Kim. 2016. "Biomarker Detection Technologies and Future Directions." *The Analyst* 141 (3). Royal Society of Chemistry: 740–55. doi:10.1039/C5AN01790D.

Nobili, Flavio, and Silvia Morbelli. 2010. "[18F]FDG-PET as a Biomarker for Early Alzheimer's Disease." *The Open Nuclear Medicine Journal* 2: 46–52. Available at: https://benthamopen.com/contents/pdf/TONMEDJ/TONMEDJ-2-46.pdf.

Pagano, G., F. Niccolini, and M. Politis. 2016. "Imaging in Parkinsons Disease." *Clinical Medicine* 16 (4): 371–75. doi:10.7861/clinmedicine.16-4-371.

Pasinszki, Tibor, Melinda Krebsz, Thanh Tran Tung, and Dusan Losic. 2017. "Carbon Nanomaterial Based Biosensors for Non-Invasive Detection of Cancer and Disease Biomarkers for Clinical Diagnosis." *Sensors (Basel, Switzerland)* 17 (8). Multidisciplinary Digital Publishing Institute (MDPI). doi:10.3390/s17081919.

Patel, Seema, Raj J Shah, Paul Coleman, and Marwan Sabbagh. 2011. "Potential Peripheral Biomarkers for the Diagnosis of Alzheimer's Disease." *International Journal of Alzheimer's Disease* 2011 (October). Hindawi: 572495. doi:10.4061/2011/572495.

Petzold, A, M J Eikelenboom, D Gveric, G Keir, M Chapman, R H C Lazeron, M L Cuzner, et al. 2002. "Markers for Different Glial Cell Responses in Multiple Sclerosis: Clinical and Pathological Correlations." *Brain : A Journal of Neurology* 125 (Pt 7): 1462–73.

Plavina, Tatiana, Meena Subramanyam, Gary Bloomgren, Sandra Richman, Amy Pace, Sophia Lee, Brian Schlain, Denise Campagnolo, Shibeshih Belachew, and Barry Ticho. 2014. "Anti-JC Virus Antibody Levels in Serum or Plasma Further Define Risk of Natalizumab-Associated Progressive Multifocal Leukoencephalopathy." *Annals of Neurology* 76 (6): 802–12. doi:10.1002/ana.24286.

Prince, Martin, Ms Adelina Comas-Herrera, Martin Knapp, Maëlenn Guerchet, and Ms Maria Karagiannidou. 2016. "World Alzheimer Report 2016 Improving Healthcare for People Living with Dementia Coverage, Quallty and Costs Now and In the Future." https://www.alz.co.uk/research/WorldAlzheimerReport2016.pdf.

"Public Health Challenges WHO Library Cataloguing-in-Publication Data." 2018. *World Health Organization*. Accessed January 8. Available at: http://www.who.int/mental_health/neurology/neurological_disorders_report_web.pdf.

Pumera, Martin, Adriano Ambrosi, Alessandra Bonanni, Elaine Lay Khim Chng, and Hwee Ling Poh. 2010. "Graphene for Electrochemical Sensing and Biosensing." *TrAC Trends in Analytical Chemistry* 29 (9). Elsevier: 954–65. doi:10.1016/J.TRAC.2010.05.011.

Regev, Keren, Anu Paul, Brian Healy, Felipe von Glenn, Camilo Diaz-Cruz, Taha Gholipour, Maria Antonietta Mazzola, et al. 2016. "Comprehensive Evaluation of Serum microRNAs as Biomarkers in Multiple Sclerosis." *Neurology(R) Neuroimmunology & Neuroinflammation* 3 (5). American Academy of Neurology: e267. doi:10.1212/NXI.0000000000000267.

Reina, Giacomo, José Miguel González-Domínguez, Alejandro Criado, Ester Vázquez, Alberto Bianco, Maurizio Prato, K. A. Dawson, et al. 2017. "Promises, Facts and Challenges for Graphene in Biomedical Applications." *Chemical Society Reviews* 46 (15). The Royal Society of Chemistry: 4400–4416. doi:10.1039/C7CS00363C.

Reitz, Christiane, and Richard Mayeux. 2014. "Alzheimer Disease: Epidemiology, Diagnostic Criteria, Risk Factors and Biomarkers." *Biochemical Pharmacology* 88 (4). NIH Public Access: 640–51. doi:10.1016/j.bcp.2013.12.024.

Rivero-Santana, Amado, Daniel Ferreira, Lilisbeth Perestelo-Pérez, Eric Westman, Lars-Olof Wahlund, Antonio Sarría, and Pedro Serrano-Aguilar. 2016. "Cerebrospinal Fluid Biomarkers for the Differential Diagnosis between Alzheimer's Disease and Frontotemporal Lobar Degeneration: Systematic Review, HSROC Analysis, and Confounding Factors." *Journal of Alzheimer's Disease* 55 (2). IOS Press: 625–44. doi:10.3233/JAD-160366.

Savage, M. J., J. Kalinina, A. Wolfe, K. Tugusheva, R. Korn, T. Cash-Mason, J. W. Maxwell, et al. 2014. "A Sensitive A Oligomer Assay Discriminates Alzheimer's and Aged Control Cerebrospinal Fluid." *Journal of Neuroscience* 34 (8): 2884–97. doi:10.1523/JNEUROSCI.1675-13.2014.

Schaffer, Cole, Nakia Sarad, Ashton DeCrumpe, Disha Goswami, Sara Herrmann, Jose Morales, Parth Patel, and Jim Osborne. 2015. "Biomarkers in the Diagnosis and Prognosis of Alzheimer's Disease." *Journal of Laboratory Automation* 20 (5). SAGE PublicationsSage CA: Los Angeles, CA: 589–600. doi:10.1177/2211068214559979.

Song, Yang, Yanan Luo, Chengzhou Zhu, He Li, Dan Du, and Yuehe Lin. 2016. "Recent Advances in Electrochemical Biosensors Based on Graphene Two-Dimensional Nanomaterials." *Biosensors & Bioelectronics* 76 (February): 195–212. doi:10.1016/j.bios.2015.07.002.

Sørensen, Per Soelberg, Poul Erik Hyldgaard Jensen, Aiden Haghikia, Malin Lundkvist, Christian Vedeler, Finn Sellebjerg, Nils Koch-Henriksen, et al. 2011. "Occurrence of Antibodies against Natalizumab in Relapsing Multiple Sclerosis Patients Treated with Natalizumab." *Multiple Sclerosis Journal* 17 (9): 1074–78. doi:10.1177/1352458511404271.

Stangel, Martin, Sten Fredrikson, Edgar Meinl, Axel Petzold, Olaf Stüve, and Hayrettin Tumani. 2013. "The Utility of Cerebrospinal Fluid Analysis in Patients with Multiple Sclerosis." *Nature Reviews Neurology* 9 (5): 267–76. doi:10.1038/nrneurol.2013.41.

Summers, Mathew J., Tamas Madl, Alessandro E. Vercelli, Georg Aumayr, Doris M. Bleier, and Ludovico Ciferri. 2017. "Deep Machine Learning Application to the Detection of Preclinical Neurodegenerative Diseases of Aging." *DigitCult - Scientific Journal on Digital Cultures* 2 (2): 9–24. doi:10.4399/97888255088952.

Sun, Meng, Huijuan Liu, Yang Liu, Jiuhui Qu, and Jinghong Li. 2015. "Graphene-Based Transition Metal Oxide Nanocomposites for the Oxygen Reduction Reaction." *Nanoscale* 7 (4). The Royal Society of Chemistry: 1250–69. doi:10.1039/C4NR05838K.

Tao L. Lowe, ‡, ‡ Andrea Strzelec, § and Laura L. Kiessling, and ‡ Regina M. Murphy*. 2001. "Structure−Function Relationships for Inhibitors of β-Amyloid Toxicity Containing the Recognition Sequence KLVFF†." American Chemical Society . doi:10.1021/BI002734U.

Terse-Thakoor, Trupti, Sushmee Badhulika, and Ashok Mulchandani. 2017. "Graphene Based Biosensors for Healthcare." *Journal of Materials Research* 32 (15): 2905–29. doi:10.1557/jmr.2017.175.

Tsang, Benjamin K-T, and Richard Macdonell. 2011. "Multiple Sclerosis- Diagnosis, Management and Prognosis." *Australian Family Physician* 40 (12): 948–55. http://www.ncbi.nlm.nih.gov/pubmed/22146321.

Vistbakka, Julia, Irina Elovaara, Terho Lehtimäki, and Sanna Hagman. 2017. "Circulating microRNAs as Biomarkers in Progressive Multiple Sclerosis." *Multiple Sclerosis Journal* 23 (3): 403–12. doi:10.1177/1352458516651141.

"World Population Ageing 2015." 2015. Available at: http://www.un.org/en/development/desa/population/publications/pdf/ageing/WPA2015_Report.pdf.

Xiao, Songhua, Daoyou Zhou, Ping Luan, Beibei Gu, Longbao Feng, Shengnuo Fan, Wang Liao, et al. 2016. "Graphene Quantum Dots Conjugated Neuroprotective Peptide Improve Learning and Memory Capability." *Biomaterials* 106 (November): 98–110. doi:10.1016/j.biomaterials.2016.08.021.

Xu, Qiao, Ho Cheng, Joshua Lehr, Amol V. Patil, and Jason J. Davis. 2015. "Graphene Oxide Interfaces in Serum Based Autoantibody Quantification." *Analytical Chemistry* 87 (1). American Chemical Society: 346–50. doi:10.1021/ac503890e.

Yu, Huitao, Bangwen Zhang, Chaoke Bulin, Ruihong Li, and Ruiguang Xing. 2016. "High-Efficient Synthesis of Graphene Oxide Based on Improved Hummers Method." *Scientific Reports* 6 (November). Nature Publishing Group: 36143. doi:10.1038/srep36143.

Zhang, Xin Y., Zhen L. Yang, Guang M. Lu, Gui F. Yang, and Long J. Zhang. 2017. "PET/MR Imaging: New Frontier in Alzheimer's Disease and Other Dementias." *Frontiers in Molecular Neuroscience* 10 (November): 343. doi:10.3389/fnmol.2017.00343.

Zhou, Chao, Sihao Chen, Jianzhong Lou, Jihu Wang, Qiujie Yang, Chuanrong Liu, Dapeng Huang, and Tonghe Zhu. 2014. “Graphene’s Cousin: The Present and Future of Graphane.” *Nanoscale Research Letters* 9 (1): 26. doi:10.1186/1556-276X-9-26.

Zhu, Junjiang, Ping Xiao, Hailong Li, and Sónia A. C. Carabineiro. 2014. “Graphitic Carbon Nitride: Synthesis, Properties, and Applications in Catalysis.” *ACS Applied Materials & Interfaces* 6 (19). American Chemical Society: 16449–65. doi:10.1021/am502925j.